Carcinogenesis vol.22 no.2 pp.279–286, 2001

Urinary benzene as a biomarker of exposure among occupationally exposed and unexposed subjects

Suramya Waidyanatha, Nathaniel Rothman[1], Silvia Fustinoni[2], Martyn T.Smith[3], Richard B.Hayes[1], William Bechtold[4], Mustafa Dosemeci[1], Li Guilan[5], Songnian Yin[5] and Stephen M.Rappaport[6]

Department of Environmental Sciences and Engineering, School of Public Health, University of North Carolina at Chapel Hill, Chapel Hill, NC 27599-7400, USA, [1]Division of Cancer Epidemiology and Genetics, National Cancer Institute, Bethesda, MD 20892, USA, [2]Istituti Clinici di Perfezionamento, Via S.Barnaba 8, I-20122 Milan, Italy, [3]School of Public Health, University of California at Berkeley, Berkeley, CA 94720, USA, [4]Lovelace Respiratory Research Institute, PO Box 5890, Albuquerque, NM 87185, USA and [5]Chinese Academy of Preventive Medicine, Institute of Occupational Medicine, Beijing, China

[6]To whom correspondence should be addressed
Email: stephen_rappaport@unc.edu

Urinary benzene (UB) was investigated as a biomarker of exposure among benzene-exposed workers and unexposed subjects in Shanghai, China. Measurements were performed via headspace solid phase microextraction of 0.5 ml of urine specimens followed by gas chromatography–mass spectrometry. This assay is simple and more sensitive than other methods (detection limit 0.016 μg benzene/l urine). The median daily benzene exposure was 31 p.p.m. (range 1.65–329 p.p.m.). When subjects were divided into controls (n = 41), those exposed to ≤31 p.p.m. benzene (n = 22) and >31 p.p.m. benzene (n = 20), the median UB levels were 0.069, 4.95 and 46.1 μg/l, respectively (Spearman r = 0.879, $P < 0.0001$). A linear relationship was observed between the logarithm of UB and the logarithm of benzene exposure in exposed subjects according to the following equation: ln(UB, μg/l) = 0.196 + 0.709 ln (exposure, p.p.m.) (r = 0.717, $P < 0.0001$). Considering all subjects, linear relationships were also observed between the logarithm of UB and the corresponding logarithms of four urinary metabolites of benzene, namely *t,t*-muconic acid (r = 0.938, $P < 0.0001$), phenol (r = 0.826, $P < 0.0001$), catechol (r = 0.812, $P < 0.0001$) and hydroquinone (r = 0.898, $P < 0.0001$). Ratios of individual metabolite levels to total metabolites versus UB provide evidence of competitive inhibition of CYP450 enzymes leading to increased production of phenol and catechol at the expense of hydroquinone and muconic acid. Among control subjects UB was readily detected with a mean level of 0.145 μg/l (range 0.027–2.06 μg/l), compared with 5.63 μg/l (range 0.837–26.38 μg/l) in workers exposed to benzene below 10 p.p.m. ($P < 0.0001$). This suggests that UB is a good biomarker for exposure to low levels of benzene.

Introduction

Benzene is an important industrial chemical that is known to cause hematotoxicity and leukemia in humans (1–3). Worldwide production of benzene was about 13.6×10^6 metric tons in 1992 (4). Since benzene is a constituent of gasoline (currently regulated at 1% in the USA) (5) and tobacco smoke (6), it is a ubiquitous environmental contaminant. Environmental exposures to benzene tends to range between 2 and 6 p.p.b. while occupational exposures in the USA range between 0.1 and 5 p.p.m. over a work day (7).

Following exposure, ~17% of benzene is exhaled (8). The remaining 83% of the absorbed benzene dose is metabolized and excreted as a variety of urinary products, including phenolic compounds (i.e. phenol, hydroquinone, catechol and trihydroxybenzene), *t,t*-muconic acid (MA) and *S*-phenylmercapturic acid (SPMA), as well as unmetabolized benzene (9).

All of the urinary metabolites have been investigated as short-term biomarkers of benzene exposure (10–15). Although the phenolic metabolites are significantly correlated with benzene exposure above ~5–10 p.p.m., they have proven to be unreliable biomarkers at lower exposure levels due to endogenous and dietary sources of the same compounds (16–20). Likewise, background levels of MA have recently been linked to sorbic acid (a food additive), cosmetics and pharmaceutical products (21,22) and the ability of individuals to metabolize benzene to MA shows significant genetic variability (23,24). Thus, of all metabolites investigated, SPMA can be regarded as the only specific biomarker of benzene exposure (25). However, current assays for SPMA either require derivatization prior to quantitation (11) or are not sufficiently sensitive to monitor low level exposures (15).

Urinary excretion of unmetabolized benzene has recently been suggested for biomonitoring because urinary benzene (UB) can be unequivocally related to benzene exposure. Ghittori *et al*. reported a significant correlation of UB with breathing zone air in workers exposed to benzene at levels as low as 0.1 p.p.m. (26). UB was also found to be most highly correlated with benzene exposure of six urinary biomarkers among workers exposed to 0.01–3.5 p.p.m. benzene in petroleum refineries (14).

Despite the promise of UB as a biomarker of benzene exposure, relatively few applications have been published, possibly due to methodological problems. The assay of UB by Ghittori *et al.* involves purging of benzene from 50 ml of urine followed by gas phase adsorption, thermal desorption and analysis by gas chromatography with flame ionization detection. This method eliminates interference from the biological matrix but is cumbersome and requires a rather large volume of urine to provide sufficient sensitivity (26,27). The headspace assay of UB by Kok and Ong, employing GC with a photoionization detector, is simpler and requires only 1 ml of urine (28). However, among subjects exposed below 0.25 p.p.m. benzene, levels of UB were at or below the limit of detection (40 ng/l urine, equivalent to 0.51 nmol/l) (14), suggesting insufficient sensitivity to monitor environmental exposure to benzene.

One of us recently applied headspace solid phase micro-

Abbreviations: EI, electron ionization; GC-MS, gas chromatography–mass spectrometry; HS-SPME, headspace solid phase microextraction; MA, *t,t*-muconic acid; SPMA, *S*-phenylmercapturic acid; UB, urinary benzene.

extraction (HS-SPME) to measure UB among persons environmentally exposed to benzene (29). Since the method is extremely simple, we wished to evaluate it further over a wide range of exposures with a study of benzene-exposed workers and controls conducted in Shanghai, China (30–33). Here we report the UB levels of 42 benzene-exposed subjects (range 1.65–329 p.p.m.) and 41 controls from that study, which were measured in 0.5 ml portions of urine. We observed that UB was highly correlated with benzene exposure as well as several urinary metabolites of benzene (31).

Materials and methods

Chemicals and supplies

[$^2H_6$]benzene and methanol (purge and trap grade) were obtained from Aldrich Chemical Co. (Milwaukee, WI). Benzene was obtained from Fluka Chemical Co. (Switzerland). NaCl was from Fisher Scientific (Pittsburgh, PA) and was heated at 200°C overnight prior to use. All SPME supplies were obtained from Supelco (Bellefonte, PA). A polydimethylsiloxane fiber (100 μm) was used for sampling benzene and [$^2H_6$]benzene from urine headspace. Two milliliter clear crimp-seal vials and caps were baked at 200°C overnight prior to use.

Subjects

Details of the study design and study subjects have been previously reported by Rothman *et al.* (30,31). Briefly, 44 benzene-exposed workers were selected from three factories in Shanghai, China (factory 1, benzene used to solubilize natural rubber; factory 2, benzene used as a solvent in the manufacture of adhesive tape; factory 3, benzene-based paint and varnish applied to wooden items). Forty-four controls were selected from a sewing machine manufacturing plant and an administrative facility in the same geographical area. The controls were frequency matched to the exposed subjects by age (5 year intervals) and gender.

Urine collection

Forty-three of 44 benzene-exposed subjects provided a spot urine sample. Although subjects in factory 1 worked ~5–6 h/day, most of them were exposed to benzene during a 2.5–3 h period immediately prior to urine sampling. Workers in factories 2 and 3 worked 8 h/day and urine was collected at the end of the work shift. Samples were aliquoted immediately, stored on dry ice and transported to a biorepository at the National Cancer Institute in the USA. Control subjects provided a spot urine sample during the clinical phase of the study. Samples were stored at –80°C for 6 years prior to analysis of benzene in urine from 42 exposed workers and 41 controls.

Exposure assessment

Individual exposures were monitored using passive personal monitors worn by each worker for a full work shift on five consecutive work days during the 1–2 week period prior to urine collection. The geometric mean of the five air measurements was used to calculate the individual median daily exposure to benzene in p.p.m. (note that 1 p.p.m. = 3.2 mg benzene/m^3 air). Individual exposures were also monitored in 37 of the 44 exposed workers on the same

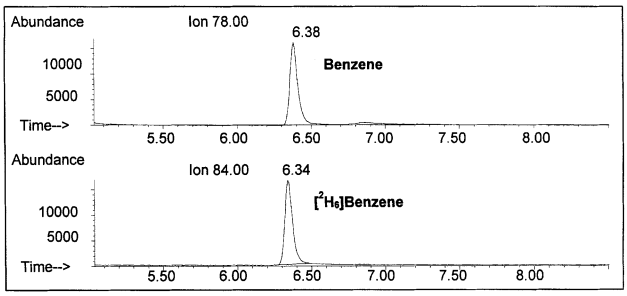

Fig. 1. GC-EI-MS selected ion monitoring chromatogram of 0.5 ml of urine from a worker exposed to 21.0 p.p.m. benzene. Ions *m/z* 78 and 84 represent benzene and [$^2H_6$]benzene, respectively. The concentration of [$^2H_6$]benzene was 10 μg/l urine.

Table I. Summary of benzene exposures and UB levels in workers occupationally exposed to benzene and controls in Shanghai, China

Group	Parameter	Benzene exposure (p.p.m.)	UB (μg/l)
Control	Mean ± SD	0.015 ± 0.018	0.145 ± 0.335
	Median (range)	0.016 (0–0.11)	0.069 (0.027–2.06)
	No. of workers	41	41
	No. of smokers	18	18
Lower exposure (⩽31 p.p.m.)	Mean ± SD	14.5 ± 8.96	8.42 ± 9.0
	Median (range)	13.6 (1.65–30.6)	4.95 (0.837–27.9)
	No. of workers	22	22
	No. of smokers	12	12
Higher exposure (>31 p.p.m.)	Mean ± SD	112 ± 76	50.2 ± 62.5
	Median (range)	92.0 (31.5–329)	46.1 (1.30–284)
	No. of workers	20	20
	No. of smokers	7	7

Table II. Least squares regression of urinary metabolites of benzene[a] on UB[b] among workers occupationally exposed to benzene and matching controls in Shanghai, China

Metabolite	Relationship[d] (Pearson *r*)	
	Exposed subjects	All subjects
Phenol	ln(phenol) = 3.2 + 0.60 ln(UB) (0.815)	ln(phenol) = 3.7 + 0.42 ln(UB) (0.826)
Catechol	ln(catechol) = 1.4 + 0.62 ln(UB) (0.815)	ln(catechol) = 2.0 + 0.39 ln(UB) (0.812)
Hydroquinone	ln(hydroquinone) = 2.1 + 0.49 ln(UB) (0.736)	ln(hydroquinone) = 2.1 + 0.48 ln(UB) (0.898)
Muconic acid	ln(MA) = 1.7 + 0.54 ln(UB) (0.730)	ln(MA) = 1.0 + 0.79 ln(UB) (0.938)

[a]Hydroquinone, phenol and MA levels were available for 43 exposed workers and 17 controls; catechol levels were available for 42 exposed workers and 16 controls.
[b]UB was measured in 42 benzene-exposed workers and 41 controls.
[c]Correlations were investigated using log transformed variates.
[d]UB levels are in μg/l and other urinary metabolites are in μg/mg creatinine.

day that urine was collected. Overall, the workers were exposed to a median value of 31 p.p.m. benzene (31). In factory 1 most workers used half-mask respirators, which were changed when the workers could detect the odor of benzene.

Control subjects in the sewing machine factory were monitored for exposure to benzene with passive monitors on 1 day only. Subjects in the administrative department were assumed to have no exposure to benzene.

Preparation of urine samples for analysis

Aliquots of urine samples from 42 exposed workers and 41 controls were stored at –80°C prior to use. Immediately prior to the assay samples were brought to room temperature, gently mixed and 0.5 ml of urine was transferred to a 2 ml vial containing 0.5 g NaCl. One microliter of 5 ng/µl [$^2H_6$]benzene in methanol was added to give a final concentration of 10 µg/l in urine and samples were immediately capped and then maintained at room temperature for 30 min.

HS-SPME

UB was measured according to the procedure of Fustinoni *et al*. (29) with the following modifications. The volume of urine was reduced from 2 to 0.5 ml. The incubation temperature during sampling of analytes from the urine headspace was increased from 40 to 45°C.

Sample vials were placed in a dry block at 45°C for 15 min to allow the analytes to come to equilibrium between the gas and liquid phases. The analytes were sampled from the vial headspace using a manual SPME assembly containing a polydimethylsiloxane fiber (10 mm, 100 µm film thickness). The fiber was housed in a stainless steel needle, which allowed penetration of the PTFE septum into the vial without damaging the fiber. Once within the vial the fiber was exposed to the headspace for 15 min, retracted and the sample was immediately analyzed by gas chromatography–mass spectrometry (GC-MS).

GC-MS analysis

Samples were analyzed on a HP 5980 series II gas chromatograph coupled to a HP 5971 A mass selective detector. A DB-5 fused silica capillary column (60 m, 0.25 mm i.d., 0.25 µm film thickness) was used with He as the carrier gas at a flow rate of 1 ml/min. A Supelco SPME injection sleeve (0.75 mm i.d.) was used as the inlet liner in the gas chromatograph. The injector and MS transfer line temperatures were 200 and 280°C, respectively. The ion source temperature was between 168 and 174°C. The GC oven was held at 50°C for 3 min and was then ramped at 8°C/min to 160°C. Late eluting compounds were removed by raising the oven temperature to 250°C. The mass spectrometer was operated in electron impact (EI) mode with electron energy set at 70 eV and ions focused at *m/z* 78 and 84 to monitor benzene and [$^2H_6$]benzene, respectively. The retention times were, respectively, 6.34 and 6.38 min for [$^2H_6$]benzene and benzene.

Standard curves were prepared by spiking urine (from a human volunteer without any known exposure to benzene) with benzene and [$^2H_6$]benzene in methanol (1 µl volumes). Standards were prepared, over the range 0–500 µg/l, sampled from the headspace and analyzed in the same manner as for the samples. Standard curves were consistently linear with $R^2 > 0.98$. Quantitation was based on peak areas relative to [$^2H_6$]benzene.

It has been shown that highly volatile organic compounds such as benzene are eliminated via the kidney by diffusion as determined by the partial pressures in urine and plasma. Consequently, the urine/blood concentration ratio should be equal to the urine/blood distribution coefficient and hence the concentration of benzene in urine should be independent of the urine output (34,35). Since a high correlation was observed between unadjusted and creatinine-adjusted UB levels (Pearson $r = 0.989$, $P < 0.0001$), UB levels were used without creatinine adjustment in subsequent analyses.

Statistical analyses

All statistical analyses were performed using SAS system software (SAS Institute, Cary, NC) using a significance level of 0.05 (two-tailed). Subjects were divided into three exposure categories, namely controls, those with geometric mean exposures <31 p.p.m. and those with geometric mean exposures <31 p.p.m. The correlation among these exposure categories and UB was tested using Spearman coefficients. In light of the highly skewed distributions, the following analyses were carried out using (natural) logarithmic transformation. Student's *t*-test was used to test for differences in UB levels between the exposed subjects and the controls as well as between smokers and non-smokers in the control group. Linear (Pearson) correlation coefficients were estimated between individual subjects' exposures and UB levels and between UB levels and previously reported urinary metabolites of benzene (phenol, catechol, hydroquinone and muconic acid) (31). Least squares linear regression was used to investigate the relationships between UB and either benzene exposure or urinary metabolite levels. Multiple regression was conducted to evaluate the impact of smoking on UB levels after adjusting for benzene exposure.

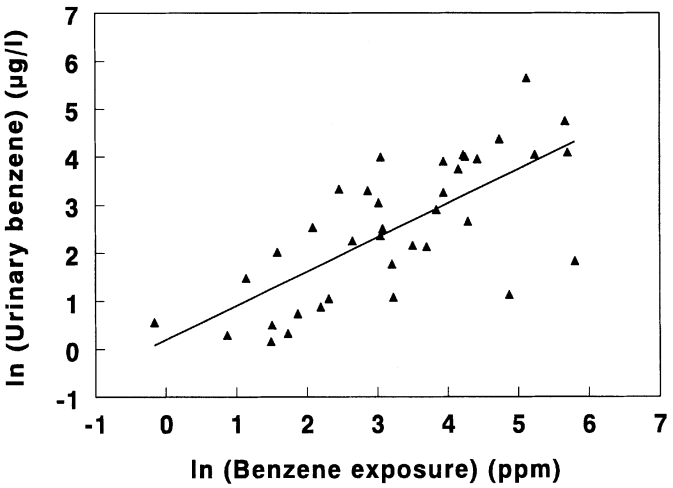

Fig. 2. Relationship between log transformed benzene exposure (p.p.m.) (on the day of urine collection) versus log transformed UB levels in workers exposed to benzene [$\ln(y) = 0.20 + 0.71 \ln(x)$, $R^2 = 0.514$]. (Exposures were unavailable for control subjects.)

Table III. Summary of smoking status and UB levels in µg/l urine in control subjects

Group	Parameter	UB (µg/l)	Cigarettes smoked per day
Non-smokers	Mean ± SD	0.116 ± 0.172	NA
	Median (range)	0.074 (0.027–0.878)	
	No. of subjects	23	
Smokers	Mean ± SD	0.191 ± 0.486	12.5 ± 5.20
	Median (range)	0.056 (0.027–2.07)	10 (4–20)
	No. of subjects	17	17

Results

Comparison between UB and benzene exposure

UB levels were measured in 42 benzene-exposed workers and 41 matched controls. A typical chromatogram obtained in GC-EI-MS in selected ion monitoring mode from a urine sample of a worker exposed to 21.0 p.p.m. benzene on the day of urine collection is shown in Figure 1. Summary statistics of benzene exposures among the three categories (controls, ⩽31 p.p.m. and >31 p.p.m.) and UB levels are shown in Table I. The median UB level among all exposed workers (9.99 µg/l) was significantly higher than that of controls (0.069 µg/l) ($P < 0.0001$). When exposed subjects were categorized into workers exposed to ⩽31 p.p.m. benzene and workers exposed to >31 p.p.m. benzene the median UB levels were 4.95 and 46.1 µg/l urine, respectively. The Spearman correlation coefficient of UB levels with respect to exposure category was 0.879 ($P < 0.0001$).

The relationship between UB and benzene exposure was also investigated on an individual basis using both the log transformed values of the individual geometric mean exposure (geometric mean of the five daily air measurements) and same day exposure (available for only 37 of the 42 subjects). (Control subjects were not included in the same day analyses because benzene exposures were not measured on the day of urine collection.) Levels of UB were significantly correlated with both the individual geometric mean exposure ($P < 0.0001$) and same day exposure ($P < 0.0001$). Although

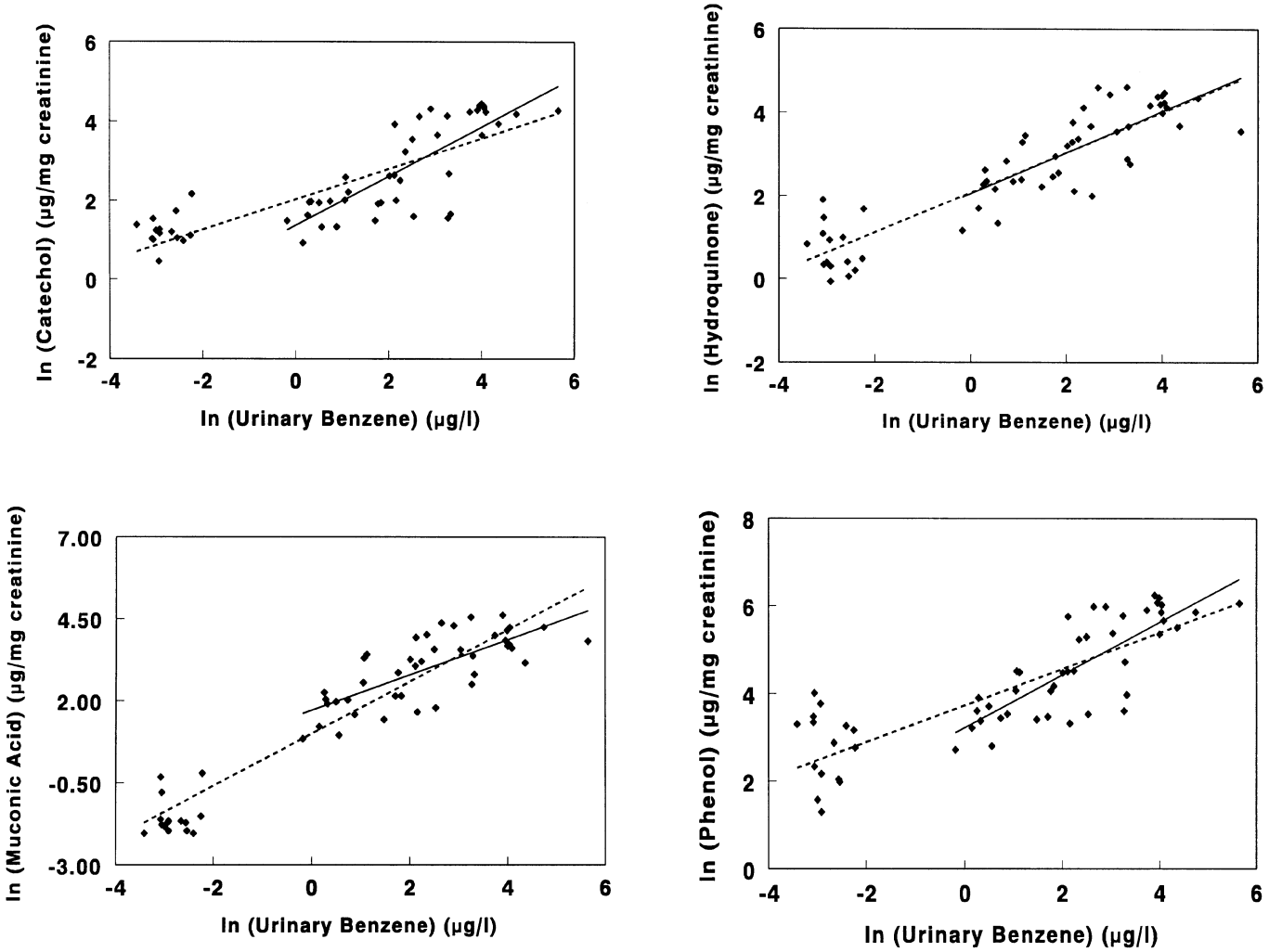

Fig. 3. Relationships between log transformed UB (µg/l) versus log transformed urinary metabolites (µg/l) in exposed and control subjects. Solid lines represent relationships in exposed subjects while dotted lines represent relationships including both control and exposed subjects.

Table IV. Summary of regressions of UB on exposure among subjects exposed to benzene in previous biomonitoring surveys and the present study

Study	Occupation	Subjects	Exposure (p.p.m.)	Exposure–UB relationship[a]	R^2
Ghittori *et al.* (27)	Chemical plants and service stations	110	0.02–4.1	$\ln(y) = 1.56 + 0.652 \ln(x)$	0.312
Ghittori *et al.* (26)	Chemical plant	124	0.01–0.5	$\ln(y) = 2.35 + 0.681 \ln(x)$	0.436
Ong *et al.* (13)	Car mechanics and shoe manufacturing	78	0.12–68	$\ln(y) = -6.42 + 0.601 \ln(x)$	0.578
Ong *et al.* (14)	Petroleum refinery	131	0.01–3.5	$\ln(y) = 0.880 + 0.640 \ln(x)$	0.250
Lagorio *et al.* (41)	Service stations	9	0.03–0.11	$\ln(y) = 7.72 + 0.423 \ln(x)$	0.568
Present study	Benzene used as a solvent in three factories	37	0.85–332	$\ln(y) = 0.20 + 0.71 \ln(x)$	0.514

[a]Note that all the relationships are expressed as (natural) logarithms of y (UB concentration, µg/l) and x (breathing zone air concentration, p.p.m.).

the correlation between UB and same day exposure (Pearson $r = 0.717$) was greater than that between UB and the geometric mean exposure (Pearson $r = 0.595$), the difference in the two correlation coefficients was not significant (Fisher's Z-test, $P = 0.831$). The scatter plot of same day benzene exposure versus UB levels is shown in Figure 2. Least squares regression revealed the following relationship: ln(UB, µg/l) = 0.20 + 0.71 ln(exposure, p.p.m.). Multiple regression analysis showed that smoking did not significantly affect the levels of UB among exposed subjects.

Comparison between UB and urinary metabolites

Of the 83 subjects, levels of phenol, hydroquinone and muconic acid were available for 38/42 exposed and 17/41 control subjects while levels of catechol were available for 37/42 exposed and 16/41 control subjects. The scatter plots of levels of UB versus metabolites are shown in Figure 3. Linear

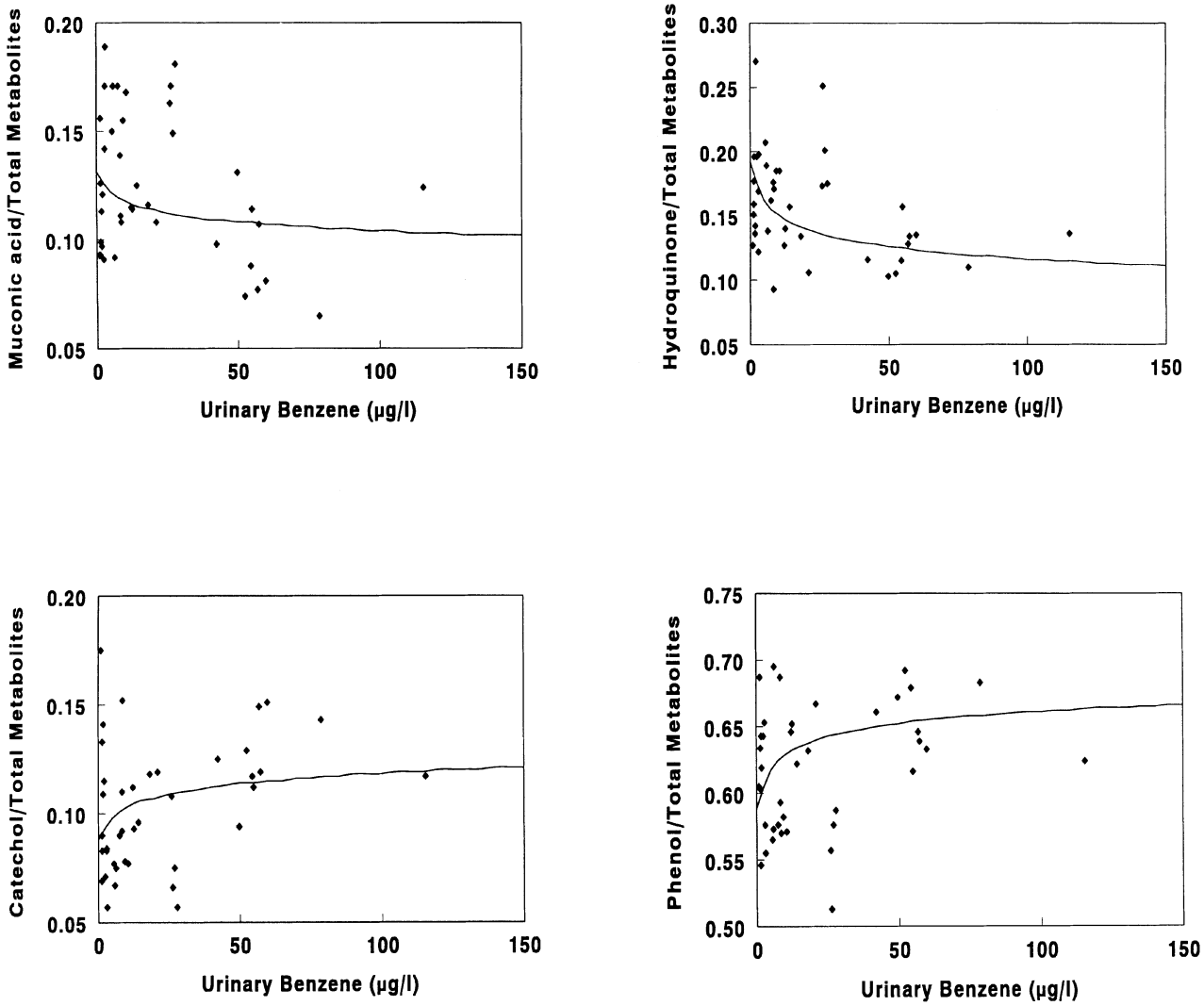

Fig. 4. Plots of ratios of individual metabolites to total metabolites versus UB levels in benzene-exposed workers. (Solid lines represent values predicted using the log–log relationships shown in Figure 3 for exposed workers only.)

relationships were found between UB and all four metabolites with or without inclusion of control subjects, as indicated in Figure 3 and summarized in Table II. All four metabolites were highly correlated with UB (Pearson $r > 0.730$ and $P < 0.001$).

Comparison of UB with respect to smoking in unexposed subjects

Forty-one control subjects including 23 non-smokers and 18 smokers were analyzed to evaluate the effect of smoking on UB. Of the 18 smoking controls all but two were from the sewing machine facility. Smoking status was available as the average number of cigarettes smoked per day in the last month. One subject reported smoking 70 cigarettes/day but had extremely low levels of UB and all urinary metabolites and hence was excluded from the analysis. Summary statistics of UB and smoking status for the 40 control subjects are shown in Table III. The median number of cigarettes smoked per day was 10 (range 4–20). The mean concentration of UB found in smokers was 191 ± 486 ng/l and in non-smokers was 116 ± 172 ng/l. No significant difference was observed between the means of the log transformed values for the two groups (Student's *t*-test, $P = 0.920$).

Discussion

The growing awareness that exposure to benzene causes leukemia has motivated development of biomarkers of exposure to this contaminant. Several urinary metabolites (phenolic metabolites, MA and SPMA) have been used to assess short-term exposures to benzene. However, their utility has been limited because of poor specificity (high background levels of the phenolic metabolites and MA) (18–22,36) lack of sensitivity (15) or complexity of the assays (SPMA) (11). Given these problems, the determination of UB presents a simple and unambiguous alternative for biological monitoring of benzene exposure. However, the routine application of UB has been limited by practical difficulties (27) and insufficient sensitivity for environmental applications (14,27).

Here we report the use of HS-SPME to measure UB among benzene-exposed workers and unexposed controls. By using a combination of headspace sampling with GC-MS this assay minimizes interference from the biological matrix and offers greatly enhanced specificity and sensitivity over previous methods. Since the limit of detection (0.016 µg/l in a 0.5 ml specimen based on a signal-to-noise ratio of 3) corresponds to a benzene exposure of 2 p.p.b., this assay can easily be applied in both occupational and environmental settings.

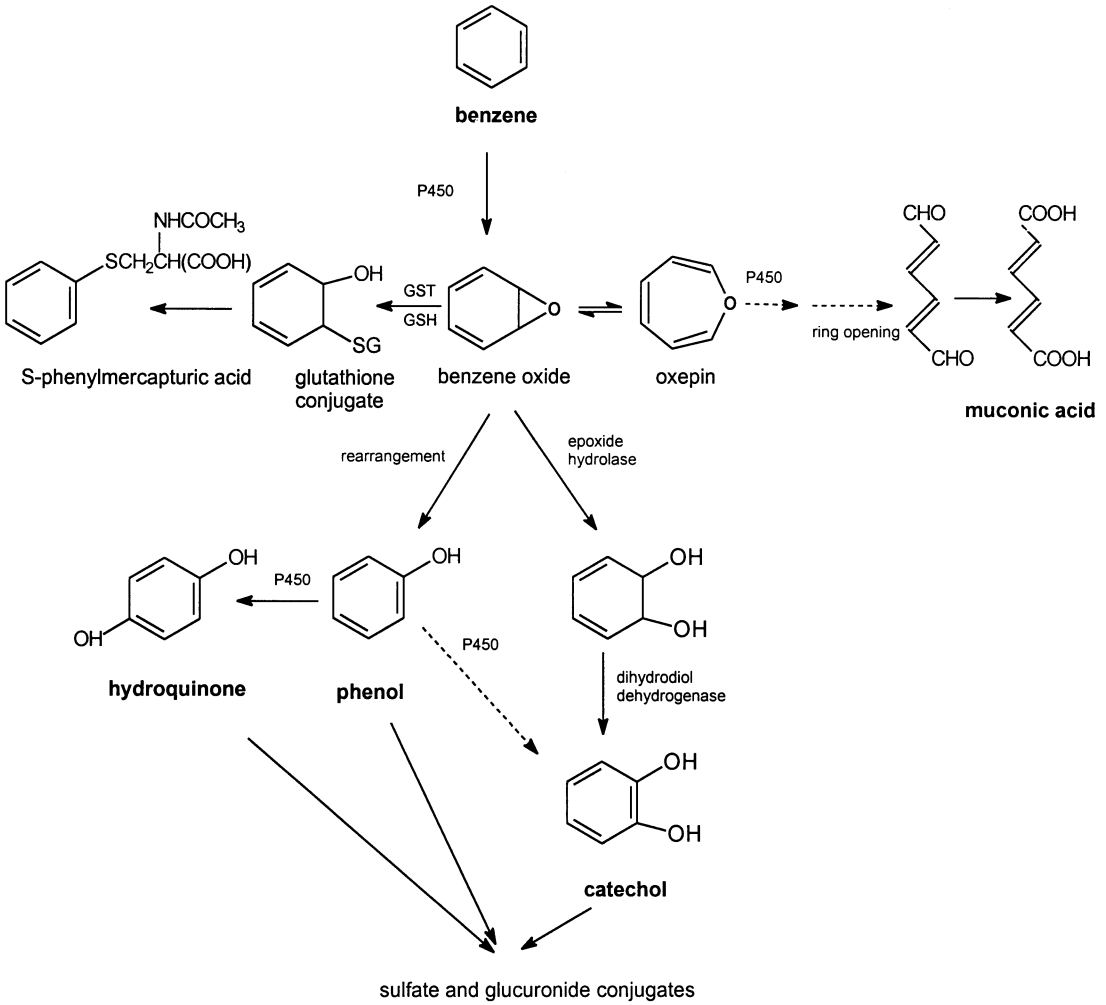

Fig. 5. The proposed metabolic scheme for benzene leading to the formation of major urinary metabolites.

The relationship between UB and benzene exposure from the current investigation is compared with previously reported results in Table IV; in each case the slope and intercept are given for the regression of ln(UB, μg/l) on ln(exposure, p.p.m.). The slopes of these log–log relationships, ranging from 0.42 to 0.71 (current investigation), suggest that levels of UB were less than proportional to benzene exposure over the ranges reported (37). This points to several possible explanations, including non-linear kinetics of uptake and metabolism of benzene, (negative) biases in the slopes due to exposure measurement errors and multiple sources of benzene exposure, such as dermal contact and cigarette smoking (37).

The relative amount of benzene excreted in urine with respect to the total amount of benzene absorbed can be estimated assuming a respiration rate of 15 ml/min and an absorption rate of 50% in the lung (8,38). Hence, at a median daily benzene exposure of 31 p.p.m. (1 p.p.m. = 3.2 mg/m^3), a worker would absorb 0.74 mg benzene/min [31 p.p.m.×3.2 mg/m^3 (p.p.m.)$^{-1}$×0.5 (retention)×15 l/min×1000 cm^3/l×1 m^3/10^6 cm^3]. Using the quantitative relationship between benzene exposure and UB in our study [ln(UB, μg/l) = 0.20 + 0.71 ln(exposure, p.p.m.)], the exposure to 31 p.p.m. benzene would lead to urinary excretion of 14 μg benzene/l urine. This translates to a rate of urinary excretion of 0.014 μg benzene/min, assuming that 1 ml of urine is removed per min (8). Thus, we estimate that at 31 p.p.m., (0.014/0.74)×100 = 1.9% of the retained benzene was excreted in urine as unmetabolized benzene. This is much lower than that accounted for by the total of benzene metabolites among these workers, which was ~80% of retained benzene (33).

Given the wide range of same day exposures among the workers in our study (0.85–332 p.p.m.), it is likely that many of the subjects experienced saturation of the CYP450 enzymes (primarily CYP4502E1) (9) responsible for metabolism of benzene and some of its metabolites. Rothman *et al.* (33) used the ratio of individual metabolite levels to total metabolites (the sum of the levels of all major metabolites, i.e. phenol, hydroquinone, catechol and MA) versus total metabolites to gauge the extent of saturable metabolism among these workers. Here we have compared the ratios of individual metabolites to total metabolites versus UB as a measure of retained benzene. As shown in Figure 4, our data point to greater than proportional production (with increasing UB) of phenol and catechol and less than proportional production of hydroquinone and MA, consistent with the earlier findings in these workers (33) and with animal experiments (39). As shown in Figure 5, these patterns suggest that benzene, phenol and benzene oxide oxepin all compete for the same cytochrome P450 enzymes. Such competitive inhibition should reduce the proportions of metabolites requiring a second oxidation, i.e. of hydroquinone (from phenol) and MA (from benzene oxide oxepin), while increasing the proportions of phenol and

catechol, both of which can be derived from benzene oxide oxepin without a second P450 oxidation. This also suggests that any possible oxidation of phenol to catechol (via CYP450), reported in the rat (40), represents a minor pathway in humans.

In our investigation UB was detected in all control subjects with a mean value of 0.145 μg/l (range 0.027–2.06 μg/l) (Table I), which was significantly lower than the mean of 5.63 μg/l (range 0.837–26.4 μg/l) among workers exposed to <10 p.p.m. benzene ($P < 0.0001$). Among controls the mean level of UB in non-smokers (0.116 μg/l) (Table III) was similar to that reported previously [i.e. 0.139 μg/l by Ghittori *et al*. (27) and 0.117 μg/l by Kok and Ong (28)]. Since there is no known endogenous production of benzene, this background of UB among non-smokers points to ambient exposure to benzene. However, among smokers (0.191 μg/l) the level of UB in our study was lower than reported elsewhere [i.e. 0.943 μg/l by Ghittori *et al*. (27) and 0.405 μg/l by Kok and Ong (28)]. This could reflect the fact that our subjects were light smokers with a median value of 10 (range 4–20) cigarettes smoked per day (Table III). In the study by Ghittori *et al.* the average number of cigarettes per day was >20 (27).

Conclusions

We here report application of the HS-SPME technique to measure UB levels among benzene-exposed workers and unexposed subjects. A significant correlation was observed between UB and benzene in breathing zone air as well as between UB and urinary benzene metabolites, i.e. MA, phenol, catechol and hydroquinone. Since the ratios of these metabolite levels to UB showed evidence of saturable CYP450 metabolism, UB can also be regarded as a measure of the total internal dose of benzene. The detection of UB in all (exposed and unexposed) subjects indicates that this assay can be used to monitor ambient as well as occupational exposure to benzene. Indeed, because UB is derived exclusively from exposure to benzene, these results suggest that UB is the optimal short-term biomarker of benzene.

Acknowledgements

The authors would like to thank Wendy McKelvey and Karen Yeowell-O'Connell for assistance with statistical analyses. This work was supported by the National Institute of Environmental Health Sciences through P42ES05948.

References

1. Cooper,K.R. and Snyder,C.A. (1988) Benzene metabolism (toxicokinetics and the molecular aspects of benzene toxicity). In Aksoy,M. (ed.), *Benzene Carcinogenecity.* CRC Press, Boca Raton, FL, pp. 35–58.
2. International Agency for Research on Cancer (1982) *IARC Monographs on the Evaluation of the Carcinogenic Risk of Chemicals to Humans. Some Inductrial Chemicals and Dyestuffs*. IARC, Lyon.
3. International Programme on Chemical Safety (1993) *Benzene—Environmental Health Criteria.* World Health Organization, Geneva, Switzerland.
4. Fishbein,L. (1992) Exposure from occupational versus other sources. *Scand. J. Work Environ. Health*, **8**, 5–16.
5. Wallace,L. (1996) Environmental exposure to benzene: an update. *Environ. Health Perspect.*, **104** (suppl. 6), 1129–1136.
6. Wallace,L., Pellizzari,E., Hartwell,T.D., Perritt,R. and Ziegenfus,R. (1987) Exposures to benzene and other volatile compounds from active and passive smoking. *Arch. Environ. Health*, **42**, 272–279.
7. Infante,P.F. (1978) Leukemia among workers exposed to benzene. *Tex. Rep. Biol. Med.*, **37**, 153–161.
8. Nomiyama,K. and Nomiyama,H. (1974) Respiratory retention, uptake and excretion of organic solvents in man. *Int. Arch. Arbeitsmed.*, **32**, 75–83.
9. Snyder,R. and Hedli,C.C. (1996) An overview of benzene metabolism. *Environ. Health Perspect.*, **104** (suppl. 6), 1165–1171.
10. Bechtold,W.E., Lucier,G., Birnbaum,L.S., Yin,S.N., Li,G.L. and Henderson,R.F. (1991) Muconic acid determinations in urine as a biological exposure index for workers occupationally exposed to benzene. *Am. Ind. Hyg. Assoc. J.*, **52**, 473–478.
11. Boogaard,P.J. and van Sittert,N.J. (1995) Biological monitoring of exposure to benzene: a comparison between S-phenylmercapturic acid, trans,trans-muconic acid and phenol. *Occup. Environ. Med.*, **52**, 611–620.
12. Boogaard,P.J. and van Sittert,N.J. (1996) Suitability of S-phenyl mercapturic acid and trans-trans-muconic acid as biomarkers for exposure to low concentrations of benzene. *Environ. Health Perspect.*, **104** (suppl. 6), 1151–1157.
13. Ong,C.N., Kok,P.W., Lee,B.L., Shi,C.Y., Ong,H.Y., Chia,K.S., Lee,C.S. and Luo,X.W. (1995) Evaluation of biomarkers for occupational exposure to benzene. *Occup. Environ. Med.*, **52**, 528–533.
14. Ong,C.N., Kok,P.W., Ong,H.Y., Shi,C.Y., Lee,B.L., Phoon,W.H. and Tan,K.T. (1996) Biomarkers of exposure to low concentrations of benzene: a field assessment. *Occup. Environ. Med.*, **53**, 328–333.
15. Inoue,O., Kanno,E., Kakizaki,M., Watanabe,T., Higashikawa,K. and Ikeda,M. (2000) Urinary phenylmercapturic acid as a marker of occupational exposure to benzene. *Ind. Health*, **38**, 195–204.
16. Bone,E., Tamm,A. and Hill,M. (1976) The production of urinary phenols by gut bacteria and their possible role in the causation of large bowl cancer. *Am. J. Clin. Nutr.*, **29**, 1448–1454.
17. Carmella,S.G., LaVoie,E.J. and Hecht,S.S. (1982) Quantitative analysis of catechol and 4-methyl catechol in human urine. *Food Chem. Toxicol.*, **20**, 587–590.
18. Deisinger,P.J., Hill,T.S. and English,C. (1996) Human exposure to natural occuring hydroquinone. *J. Toxicol. Environ. Health*, **47**, 31–46.
19. Marga,G.A. (1978) Simple phenol and phenolic compounds in food flavor. *CRC Crit. Rev. Food Sci. Nutr.*, **4**, 323–372.
20. Smith,E.A. and Macfarlane,G.T. (1996) Enumeration of human colonic bacteria producing phenolic and indolic compounds: effects of pH, carbohydrate availability and retention time on dissimilatory aromatic amino acid metabolism. *J. Appl. Bacteriol.*, **81**, 288–302.
21. Ducos,P., Gaudin,R., Robert,A., Francin,J.M. and Marie,C. (1990) Improvement in HPLC analysis of urinary trans-trans muconic acid, a promising substitute for phenol in the assesment of benzene exposure. *Int. Arch. Occup. Environ. Health*, **62**, 529–534.
22. Westöö,G. (1964) On the metabolism of sorbic acid in the mouse. *Acta Chem. Scand.*, **18**, 1373–1378.
23. Gobba,F., Rovesti,S., Borella,P., Vivoli,R., Caselgrandi,E. and Vivoli,G. (1997) Inter-individual variability of benzene metabolism to trans,trans-muconic acid and its implications in the biological monitoring of occupational exposure. *Sci. Total Environ.*, **199**, 41–48.
24. Johnson,E.S. and Lucier,G. (1992) Perspectives on risk assessment impact of recent reports on benzene. *Am. J. Ind. Med.*, **21**, 749–757.
25. van Sittert,N.J., Boogaard,P.J. and Beulink,G.D.J. (1993) Application of the urinary S-phenylmercapturic acid test as a biomarker for low levels of exposure to benzene in industry. *Br. J. Ind. Med.*, **50**, 460–469.
26. Ghittori,S., Maestri,L., Fiorentino,M.L. and Imbriani,M. (1995) Evaluation of occupational exposure to benzene by urinalysis. *Int. Arch. Occup. Environ. Health*, **67**, 195–200.
27. Ghittori,S., Fiorentino,M.L., Maestri,L., Cordioli,G. and Imbriani,M. (1993) Urinary excretion of unmetabolized benzene as an indicator of benzene exposure. *J. Toxicol. Environ. Health*, **38**, 233–243.
28. Kok,P.W. and Ong,C.N. (1994) Blood and urinary benzene determined by headspace gas chromatography with photoionization detection: application in biological monitoring of low-level nonoccupational exposure. *Int. Arch. Occup. Environ. Health*, **66**, 195–201.
29. Fustinoni,S., Giampiccolo,R., Pulvirenti,S., Buratti,M. and Colombi,A. (1999) Headspace solid-phase microextraction for the determination of benzene, toluene, ethylbenzene and xylenes in urine. *J. Chromatogr. B Biomed. Sci. Appl.*, **723**, 105–115.
30. Rothman,N., Smith,M.T., Hayes,R.B., Li,G.L., Irons,R.D., Dosemeci,M., Haas,R., Stillman,W.S., Linet,M., Xi,L.Q., Bechtold,W.E., Wiemels,J., Campleman,S., Zhang,L., Quintana,P.J., Titenko-Holland,N., Wang,Y.Z., Lu,W., Kolachana,P., Meyer,K.B. and Yin,S. (1996) An epidemiologic study of early biologic effects of benzene in Chinese workers. *Environ. Health Perspect.*, **104** (suppl. 6), 1365–1370.
31. Rothman,N., Li,G.L., Dosemeci,M., Bechtold,W.E., Marti,G.E., Wang,Y.Z., Linet,M., Xi,L.Q., Lu,W., Smith,M.T., Titenko-Holland,N., Zhang,L.P., Blot,W., Yin,S.N. and Hayes,R.B. (1996) Hematotoxicity among Chinese workers heavily exposed to benzene. *Am. J. Ind. Med.*, **29**, 236–246.
32. Rothman,N., Smith,M.T., Hayes,R.B., Traver,R.D., Hoener,B., Campleman,S., Li,G.L., Dosemeci,M., Linet,M., Zhang,L., Xi,L., Wacholder,S., Lu,W., Meyer,K.B., Titenko-Holland,N., Stewart,J.T., Yin,S. and Ross,D. (1997) Benzene poisoning, a risk factor for hematological

malignancy, is associated with the NQO1 609C→T mutation and rapid fractional excretion of chlorzoxazone. *Cancer Res.*, **57**, 2839–2842.
33. Rothman,N., Bechtold,W.E., Yin,S.N., Dosemeci,M., Li,G.L., Wang,Y.Z., Griffith,W.C., Smith,M.T. and Hayes,R.B. (1998) Urinary excretion of phenol, catechol, hydroquinone and muconic acid by workers occupationally exposed to benzene. *Occup. Environ. Med.*, **55**, 705–711.
34. Boeniger,M.F., Lowry,L.K. and Rosenberg,J. (1993) Interpretation of urine results used to assess chemical exposure with emphasis on creatinine adjustments: a review. *Am. Ind. Hyg. Assoc. J.*, **54**, 615–627.
35. Rosenberg,J., Fiserova-Bergerova,V. and Lowry,L.K. (1989) Biological monitoring IV. Measurement in urine. *Appl. Ind. Hyg.*, **4**, F16–F20.
36. Pezzagno,G., Maestri,L. and Fiorentino,M.L. (1999) Trans,trans-muconic acid, a biological indicator to low levels of environmental benzene: some aspects of its specificity. *Am. J. Ind. Med.*, **35**, 511–518.
37. Rappaport,S.M., Symanski,E., Yager,J.W. and Kupper,L.L. (1995) The relationship between environmental monitoring and biological markers in exposure assessment. *Environ. Health Perspect.*, **103** (suppl. 3), 49–53.
38. Inoue,O., Seiji,K., Nakatsuka,H., Watanabe,T., Yin,S., Li,G.L., Cai,S.X., Jin,C. and Ikeda,M. (1989) Excretion of 1,2,4-benzenetriol in the urine of workers exposed to benzene. *Br. J. Ind. Med.*, **46**, 559–565.
39. Sabourin,P.J., Bechtold,W.E., Griffith,W.C., Birnbaum,L.S., Lucier,G. and Henderson,R.F. (1989) Effect of exposure concentration, exposure rate and route of administration on metabolism of benzene by F344 rats and B6C3F1 mice. *Toxicol. Appl. Pharmacol.*, **99**, 421–444.
40. Schlosser,P.M., Bond,J.A. and Medinsky,M.A. (1993) Benzene and phenol metabolism by mouse and rat liver microsomes. *Carcinogenesis*, **14**, 2477–2486.
41. Lagorio,S., Crebelli,R., Ricciarello,R., Conti,L., Iavarone,I., Zona,A., Ghittori,S. and Carere,A. (1998) Methodological issues in biomonitoring of low level exposure to benzene. *Occup. Med.*, **48**, 497–504.

Received July 26, 2000; revised October 12, 2000;
accepted October 16, 2000